[CANCER RESEARCH 62, 1030–1035, February 15, 2002]

Circulating Insulin-like Growth Factor-I Levels Regulate Colon Cancer Growth and Metastasis

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ABSTRACT

It has been shown previously that slight elevations in serum levels of insulin-like growth factor-I (IGF-I) are correlated with an increased risk for developing prostate, breast, colon, and lung cancer. The aim of this study was to determine the role of serum IGF-I levels in the process of stimulating tumor growth and metastasis in a mouse model of colon cancer. Colon 38 adenocarcinoma tissue fragments were orthotopically transplanted by attachment to the surface of the cecum in control and liver-specific IGF-I-deficient (LID) mice in which serum IGF-I levels are 25% of that in control mice. A total of 156 male mice at 5 weeks of age (74 control mice and 82 LID mice) received tumor transplants. Mice were divided randomly into two groups; one group was injected i.p. with recombinant human IGF-I (2 mg/kg) twice daily for 6 weeks, and the other group received saline injections. IGF-I treatment increased the serum levels of IGF-I and IGFBP-3 in both control and LID mice. In the saline-injected group, the incidence of tumor growth on the cecum as well as the frequency of hepatic metastasis was significantly higher in control mice as compared with LID mice. Both control and LID mice treated with recombinant human IGF-I displayed significantly increased rates of tumor development on the cecum and metastasis to the liver, as compared with saline-injected mice. The number of metastatic nodules in the liver was significantly higher in control mice as compared with LID mice. The expression of vascular epithelial growth factor (VEGF) as well as vessel abundance in the cecum tumors was dependent on the levels of serum IGF-I. This study supports the hypothesis that circulating IGF-I levels play an important role in tumor development and metastasis.

INTRODUCTION

The potential role of the IGFs[2] (IGF-I and IGF-II) in cancer cell growth has been widely investigated. The IGFs are expressed ubiquitously and act as endocrine, paracrine, and autocrine growth factors. The IGFs activate the IGF-IR, which is frequently overexpressed in cancer cells. The activated IGF-IR, like other cell surface tyrosine kinase receptors, initiates a number of intracellular signaling cascades, which enhance progression of the cell cycle and inhibit apoptosis. These effects result in an accumulation of cancer cells. There are six known IGFBPs, which were also shown to affect cancer cell growth by modulating the interactions between the IGFs and the IGF-IR. The IGFBPs also have certain ligand-independent actions, although the mechanism(s) of these effects has not yet been established.

When rhIGF-I is administered to nude mice with s.c. fibrosarcomas, tumor growth is enhanced, and the latency period for tumor development is shortened (1). In this study, IGF-I treatment increased circulating levels of IGF-I, and IGF-I levels in the peripheral tissues were presumably increased as well. In other murine cancer models, it has been shown that cancer growth may be retarded by food deprivation, which lowers circulating IGF-I levels. A reversible effect has also been shown by IGF-I treatment of the same food-restricted mice (2).

Recent epidemiological studies have established a correlation between circulating levels of IGF-I and IGFBP-3 and the relative risk for developing colon, breast, prostate, and lung cancer (3–7). Ma *et al.* (3) reported that higher levels of IGF-I and lower levels of IGFBP-3 are independently associated with an increased risk of colorectal cancer. Hankinson's results indicated that plasma IGF-I concentrations may help to identify women at high risk of developing breast cancer (4). Furthermore, in a recent study, subjects with adenomas that were designated as high risk for developing into cancer had significantly higher IGF-I levels and significantly lower IGFBP-3 levels than did subjects with normal colonoscopy examinations or those with adenomas that were designated as low risk (8). Although a direct causative relationship has not yet been established, these findings have led investigators to question whether circulating levels of IGF-I and IGFBP-3 may indeed play a role in the growth of tumors. In addition, patients with acromegaly who have elevated serum levels of growth hormone and IGF-I may be at increased risk for developing colonic premalignant polyps and cancer (9).

This study was aimed to specifically determine the role of circulating IGF-I levels in tumor growth and metastasis (*i.e.*, in the absence of alterations in peripheral *IGF-I* gene expression). To this end, we used the LID mouse model that was created using the Cre-lox/P system (10). We have shown previously that LID mice exhibit a 75% reduction in circulating IGF-I levels, whereas IGF-I expression in peripheral (nonhepatic) tissues is not different from that in control mice (10). Murine colon 38 adenocarcinoma tissue fragments were transplanted onto the cecum of LID and control mice at the age of 5–6 weeks. Mice were treated with rhIGF-I or saline for 6 weeks after tumor transplantation. The growth of cecum tumors and the extent of hepatic metastases were measured after 6 weeks of treatment. Interestingly, the incidence of cecum tumor growth and hepatic metastases was significantly higher in control mice, as compared with LID mice treated with saline. Administration of rhIGF-I increased tumor growth and metastases in both control and LID mice. These results suggest that circulating IGF-I levels play an important role in tumor growth.

MATERIALS AND METHODS

Animal Husbandry and PCR Genotyping. The generation of LID mice has been described previously (10). The control mice used in these studies express the fourth exon of the *IGF-I* gene flanked by two LoxP sites [designated as LL− in the work of Yakar *et al.* (10)]. The LID mice express the fourth exon of the *IGF-I* gene flanked by two LoxP sites, but these animals also express the *Cre* transgene exclusively in the liver, under the control of the albumin/enhancer promoter sequence [LL+ in the work of Yakar *et al.* (10)]. Animals were genotyped using PCR on tail DNA, as described elsewhere (11). All procedures were approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases, NIH.

Mouse Model of Colon Carcinoma Growth and Hepatic Metastasis. Colon 38, a mouse adenocarcinoma created in C57BL/6 mice (kindly provided by Dr. Camalier of the National Cancer Institute, NIH, Frederick, MD) is maintained by serial s.c. inoculation in female C57BL/6 mice. Tumor expansion and processing were performed as described previously (12). Colon carcinoma growth and hepatic metastases were evaluated in mice, as described

Received 5/22/01; accepted 12/17/01.

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2 The abbreviations used are: IGF, insulin-like growth factor; IGF-IR, IGF-I receptor; IGFBP, IGF binding protein; rhIGF, recombinant human IGF; LID, liver-specific IGF-I-deficient; VEGF, vascular endothelial growth factor.

previously (13) with a slight modification. Briefly, mice were anesthetized by s.c. injection of 2.5% Avertin at a dose of 10 μl/g body weight. The abdominal area was shaved and sterilized, and an incision was made in the middle left abdomen. The cecum was gently exposed, and one piece of the Colon 38 tumor tissue ($4 \times 4 \times 4$ mm^3 in size and weighing 45 ± 0.68 mg) was sutured on the surface of the cecum using 9-0 sutures (Ethicon, Somerville, NJ). The cecum was then returned to the abdominal cavity. The incision was closed in two layers; the peritoneum and muscle were closed using catgut sutures, and the skin was closed with stainless steel wound clips.

IGF-I Injections. Starting on the second day after surgery, mice were injected twice daily i.p. with 2 mg/kg of rhIGF-I (Genentech, San Francisco, CA) or with the same volume of saline. Mice were euthanized after 6 weeks of injections, and the cecum and liver were removed. The weight of the cecum tumor and the number of visible hepatic metastatic nodules on the surface of liver were recorded.

RNase Protection Assay. *IGF-IR* gene expression levels were determined by RNase Protection Assays in the cecum tumor and in metastatic nodule(s) in the liver. Tissues were homogenized with a Polytron homogenizer (Brinkmann Instruments, Wesbury, NY) in RNazol B reagent (Tel-Test, Inc., Friendswood, TX), and total RNA was isolated according to the manufacturer's instructions. RNase protection assays were carried out as described previously (10). Briefly, 50 μg of total RNA were hybridized overnight at 45°C with ^{32}P-labeled riboprobes for exon 3 of the IGF-IR and β-actin (Ambion, Austin, TX). Protected bands were denatured and separated on 8% polyacrylamide gels. The resulting gels were dried and exposed to X-Omat MS film overnight. The protected bands corresponding to IGF-IR and β-actin mRNA were quantified by Phosphorimager (Fuji Film, BSA-1800 II).

Western Blot Analysis. Tissues were homogenized in lysis buffer [150 mM sodium chloride, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP40, 100 mM sodium fluoride, 10 mM sodium PP_i, 10 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride, protease inhibitors (Roche, Indianapolis, IN) 1 tablet/10 ml buffer]. Samples were solubilized for 30 min on ice, centrifuged at 600 rpm for 20 min at 4°C to remove crude particulate matter, and then centrifuged at 40,000 rpm for 45 min at 4°C in a Beckman Ti-70 rotor. Protein (200 μg) from the resulting supernatants was subjected to 8% SDS-PAGE (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes (Invitrogen). The blots were blocked with 5% insulin-free BSA and incubated with an anti-IGF-IRα antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and followed by secondary peroxidase-conjugated donkey antirabbit antibody (Amersham Pharmacia Biotech, Piscataway, NJ). IGF-IR immunoreactivity was detected by chemiluminescence (New England Nuclear, Boston, MA) and quantified by scanning the radiographs and then analyzing the bands with the MacBas version 2.52 software (Fuji Photo Film USA, Inc.).

Measurement of IGF-I and IGFBP-3. Serum concentration of IGF-I were measured in duplicates in blood samples of both IGF-I- and saline-treated mice (control and LID) by RIA (National Hormone and Pituitary Program, NIDDK). Sensitivity of the assay was 0.02 ng/ml. IGFBP-3 levels were analyzed using a ligand blot assay as reported previously (14) with slight modifications. Briefly, 2 μl of serum were electrophoresed through a 4–12% SDS-PAGE (Invitrogen). Proteins were transferred to nitrocellulose membranes (Invitrogen). The blots were incubated overnight in TBS containing 2% of BSA. 1.5 million cpm of ^{125}I-labeled IGF-I were added to each membrane overnight. Blots were washed twice with TBS containing 0.1% Tween 20, twice with TBS, and then exposed to Phosphorimager (Fuji Film BAS-1800 II) for quantification.

Tumor Morphology and Immunohistochemical Staining. Tumor tissue was removed and fixed in 4% paraformaldehyde. Tissues were then embedded in paraffin, and consecutive 5-μm sections were cut and mounted on glass slides. Sections were stained with H&E. Slides were also prepared on poly-L-lysine coated for immunohistochemical staining. An anti-VEGF rabbit polyclonal antibody, obtained from Santa Cruz Biotechnology, was used at a dilution of 1:150. A rabbit polyclonal antibody (Dako Co., Carpinteria, CA) was used at a 1:250 dilution for factor VIII. Immunoperoxidase staining was performed according to the manufacturer's recommended protocol (Vector ABC kit; DAB substrate kit; Vector, Inc). For positive controls, human breast cancer sections (provided by the Department of Pathology, NIH, Bethesda, MD) were stained for VEGF as well as factor VIII. Negative controls used all reagents except primary antibodies. The intensity of VEGF staining in tumor cells was graded on a scale of 0 to +3, with 0 indicating no detectable staining and +3 indicating the strongest staining, either on primary tumors (in the cecum) or metastatic tumors (in the liver). Vessel counting was performed as described previously (15).

Statistical Analyses. Differences in mean frequencies of cecum tumor growth, cecum tumor weight, hepatic metastasis, and the number of metastatic nodule(s) were analyzed by χ^2 analysis. Differences in mean serum levels of IGF-I, IGFBP-3, and mean vessel counts were analyzed by Student's *t* test. SigmaPlot5.0 software was used for all analyses.

RESULTS

The Incidence of Cecum Tumor Growth and Hepatic Metastasis Was Significantly Higher in Control Mice Compared with LID Mice. A total of 156 mice were analyzed for tumor growth. As shown in Table 1, 25 of 44 control mice (56.8%) had a palpable tumor on the cecum detectable from the fourth week after surgery. In contrast, palpable tumors on the cecum were observed in only 16 of 51 LID mice (31.3%; $P < 0.01$). The latency period until appearance of a palpable mass was significantly shorter in control mice than that of LID mice (23.1 ± 0.57 days *versus* 27.4 ± 0.76 days; $P < 0.05$). Cecum tumors in control mice were significantly larger than those in LID mice (1.57 ± 0.44 g in control mice *versus* 1.18 ± 0.37 g in LID mice; $P < 0.01$; Table 1). Furthermore, the frequency of hepatic metastasis was significantly lower in LID mice, as compared with control mice (31.3% in LID mice *versus* 44.0% in control mice; $P < 0.05$; Table 2). Fewer metastatic nodules were found in the liver of LID mice than in control mice (1.20 ± 0.45 *versus* 2.18 ± 0.60; $P < 0.01$; Table 2).

Administration of rhIGF-I Stimulates Tumor Growth and Metastasis. As shown in Tables 1 and 2**,** rhIGF-I administration increased the frequency of cecum tumor growth from 56.8 to 76.7% ($P < 0.01$) in control mice. In addition, the frequency of hepatic metastasis increased from 44 to 56.5% ($P < 0.05$) in control mice. In LID mice, rhIGF-I treatment also dramatically increased the frequency of cecum tumor growth and hepatic metastasis from 31.3 to 64.5% ($P < 0.01$) and from 31.25 to 45% ($P < 0.05$), respectively. The weight of cecum tumors was significantly increased in response to rhIGF-I in both control and LID mice, as shown in Table 1. Moreover, rhIGF-I treatment significantly increased the number of hepatic metastatic nodules in control mice, as compared with that in LID mice treated with saline (Table 2). The latency period until appearance of a palpable mass after rhIGF-I treatment was significantly shorter in control mice than that of LID mice (21.1 ± 0.31 days *versus* 24.3 ± 0.46 days; $P < 0.05$). As shown in Fig. 1, serum levels of IGF-I in LID were markedly decreased (by 75%) as compared with control mice (Fig. 1*A*). After injection of rhIGF-I, there was an increase in circulating IGF-I in control and LID mice. Similarly, IGFBP-3 levels are markedly reduced in the LID mice (by 60%); however, after rhIGF-I injection, there is an increase in the levels of IGFBP-3 in both control and LID mice.

Tumor Histology. H&E staining of consecutive paraffin sections revealed that orthotopic transplanted cecum tumors grew and invaded

Table 1 *Incidence of cecum tumor growth and cecum tumor weight*

Treatment	*n*	No. of mice displaying cecum tumor growth (%)	Average cecum tumor weight (g) ± SE
Control + saline	44	25 (56.8)[a]	1.57 ± 0.44[a]
Control + IGF-I	30	23 (76.7)[b]	2.69 ± 0.73[b]
LID + saline	51	16 (31.3)[c]	1.18 ± 0.37[c]
LID + IGF-I	31	20 (64.5)	1.65 ± 0.42

[a] Control + saline *versus* LID + saline, $P < 0.01$.
[b] Control + saline *versus* Control + IGF-I, $P < 0.01$.
[c] LID + saline *versus* LID + IGF-I, $P < 0.01$.

Table 2 *Incidence of hepatic metastasis and number of metastatic nodules*

Treatment	*n*	No. of mice displaying hepatic metastases (%)	Average number of nodules per liver ± SE
Control + saline	25	11 (44)[a]	2.18 ± 0.60[a]
Control + IGF-I	23	13 (56.5)[b]	4.92 ± 2.90[b]
LID + saline	16	5 (31.25)[c]	1.20 ± 0.45
LID + IGF-I	20	9 (45)	1.56 ± 0.53

[a] Control + saline *versus* LID + saline, $P < 0.05$.
[b] Control + saline *versus* Control + IGF-I, $P < 0.05$.
[c] LID + saline *versus* LID + IGF-I, $P < 0.05$.

all layers of the cecum in both control and LID mice, treated either with rhIGF-I or saline. Invasion of the muscularis, vascular invasion into submucosal veins, and venous emboli can be seen in some samples (Fig. 2, *A–E*). Compared with panels *A*, *B*, and *C* (Fig. 2), most parts of muscularis were still intact in the sample from a tumor obtained from a saline-treated LID mouse (Fig. 2*D*). No obvious ulceration of the mucosa was seen. Extensive necrosis can be seen both in some cecum tumors and liver metastatic tumors. The hepatic metastatic tumor (Fig. 2*F*) displayed similar histological characteristics as the primary cecum tumor (based on H&E staining). Tumor cells were immunoreactive for VEGF and were observed surrounding blood vessels. The intensity of VEGF staining was higher in the cecum tumors of control mice than in LID mice (Fig. 3, *B versus D*). Exogenous administration of rhIGF-I increased VEGF expression in both control mice and in LID mice (Fig. 3, *A versus B* for control mice; Fig. 3, *C versus D* for LID mice, respectively). Areas of neovascularization were found in all cecum tumors but were most frequent at the margins of the invasive tumor and in the central regions of tumor where necrosis can be seen. In correspondence, the vessel count (Fig. 4) was higher in the cecum tumors of control mice than that of LID mice (19.2 ± 1.01 *versus* 9 ± 1.00; $P < 0.01$). IGF-I treatment enhanced vessel count for both control mice (28.5 ± 1.06 *versus* 19.2 ± 1.01; $P < 0.01$) and LID mice (20 ± 1.70 *versus* 9 ± 1.00; $P < 0.01$).

Expression of the IGF-I Receptor in Tumor Cells. IGF-IR mRNA expression was analyzed by RNase protection assay, as detailed in "Materials and Methods." All of the cecum tumor samples in both control and LID mice expressed IGF-IR mRNA. Injection of rhIGF-I did not significantly alter IGF-IR mRNA expression levels in the cecum tumor tissue when compared with that of mice treated with saline. A small amount of IGF-IR mRNA was detected in total RNA extracted from livers containing metastatic nodules (Fig. 5). Because liver itself does not express detectable levels of IGF-IR, this indicates that the nodules have metastasized from the cecum to the liver. IGF-IR protein levels in cecum tumor tissue did not differ significantly between control and LID groups, treated or untreated with IGF-I (data not shown).

DISCUSSION

Orthotopic transplantation of colon 38 in the cecum leads to a high degree of tumor growth in the cecum by 14 days after transplantation. Liver tumor metastases are detected 21 days after transplantation to the cecum (13). In the present study, we transplanted colon 38 adenocarcinoma tissue fragments onto the cecum of LID mice and control mice to study the effects of reduced circulating IGF-I levels on tumor growth and the subsequent appearance of metastases. The genetic background of the mice used was a mixed background of 129sv, C57BL/6, and FVB/N. Despite this mixed genetic background, both control and LID mice developed cecum tumors and liver metastases, suggesting that the innate immunity that remained after multiple matings and breeding of these mice may have been less effective in rejecting cells from a pure C57BL/6 background. Indeed, our data showed that macroscopic palpable cecum tumors were present around 23 days after transplantation. This time course of tumor development is only slightly delayed, as compared with the published experiments in syngeneic mice.

When colon 38 adenocarcinoma tissue fragments were transplanted into the cecum of LID and control mice, it became apparent that significantly fewer LID mice developed tumors. Thus, we hypothesize that the lower levels of total circulating IGF-I in LID mice impaired tumor development in these animals. However, we cannot exclude the possibility that parallel reductions in IGFBP-3 or IGFBP-1 levels in LID mice may cause fewer tumors to develop. This possibility appears to be remote, because many studies have shown that IGFBP-3 inhibits tumor growth and increases apoptosis in a manner that is often independent of IGF-I. However, there are also some reports that IGFBP-3 can enhance cellular proliferation (16).

In the established model of cecum tumor growth and development, liver metastases are normally detected as early as day 21 after transplantation, with a maximum event at about 28 days after tumor transplantation (13). In preliminary experiments, we determined that 42–48 days after transplantation was the period at which the appearance of liver metastases was maximal (data not shown). Therefore, mice were sacrificed at 42–48 days after tumor transplantation in this study. The transplanted tumors grew and invaded all layers of the cecum and colon from the exterior through to the mucosal wall. Interestingly, in addition to the finding that LID mice developed fewer tumors, the sizes of the tumors that did develop were significantly smaller than those seen in control mice. The latency period until a palpable mass was detected was significantly shorter in control compared with LID mice. These observations suggest that the total serum IGF-I levels might affect the rate of tumor growth. Similar to the development of cecal tumors, the presence of visible metastases on the surface of liver occurred at a significantly lower rate in LID mice than in control mice. Importantly, the number of liver metastatic nodules

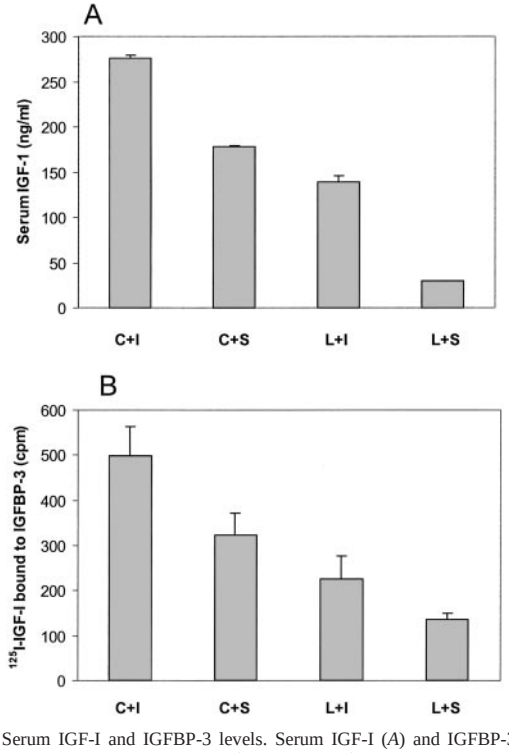

Fig. 1. Serum IGF-I and IGFBP-3 levels. Serum IGF-I (*A*) and IGFBP-3 (*B*) were measured in control (*C*) and LID mice after 6 weeks of treatments with rhIGF-I (*I*) or saline (*S*).

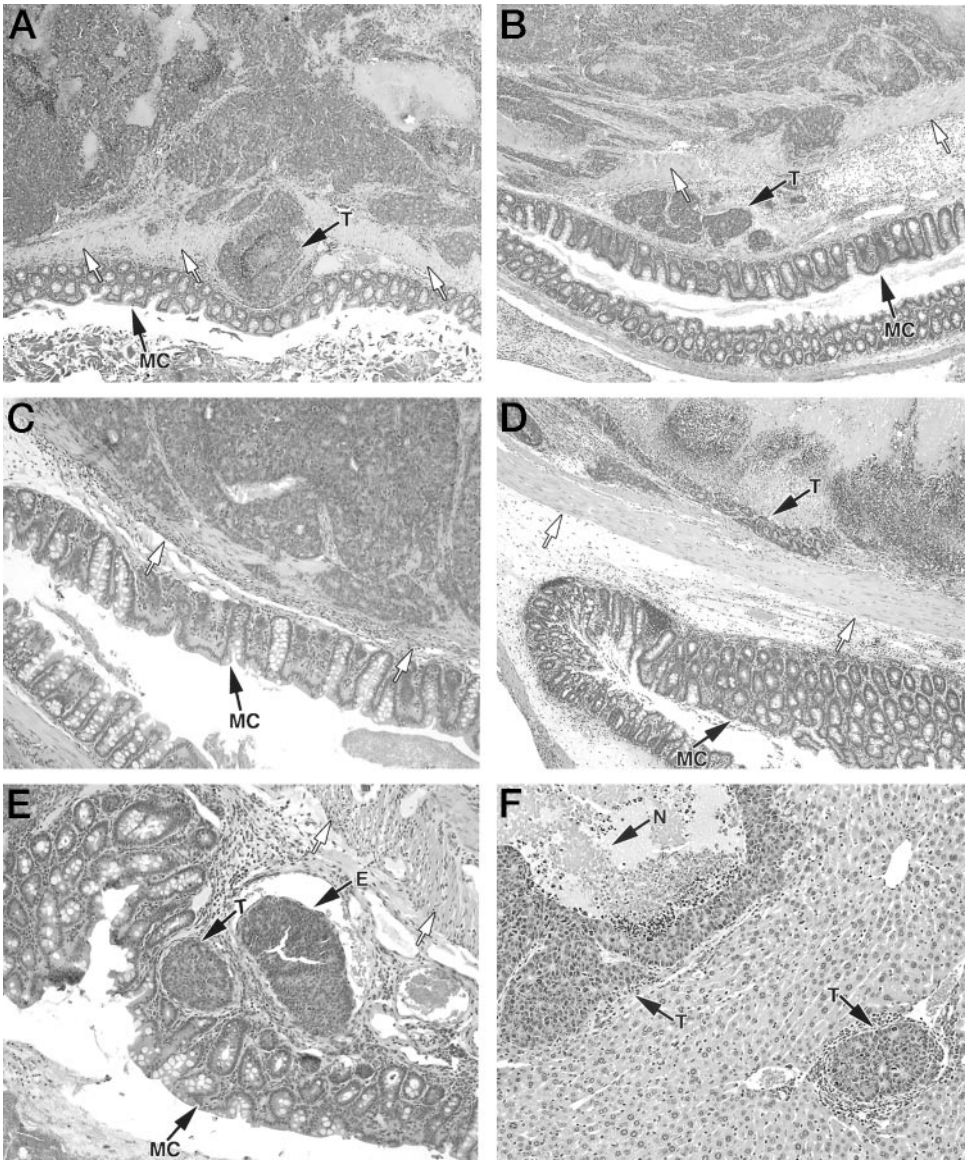

Fig. 2. Cecum tumor growth and hepatic metastasis. H&E staining shows that transplanted cecum tumors grew and invaded all layers of the cecum in both control and LID mice, treated either with rhIGF-I or saline. Invasion of the muscularis and vascular invasion into submucosal veins can be seen in some samples (*A–E*). *A*, control, IGF-I-treated; *B*, control, saline-treated; *C*, LID, IGF-I-treated; *D*, LID, saline-treated). In *D*, most parts of muscularis were still intact. Venous emboli is seen in some samples (*E*). *F*, multiple hepatic metastases. *White arrows*, muscularis. *MC*, mucosa; *T*, tumor tissue; *E*, emboli; *N*, necrosis.

was also lower in LID mice, as compared with control mice. These data suggest that circulating IGF-I levels have independent effects on the metastatic potential.

All cecum tumor specimens and liver metastases expressed IGF-IR mRNA, although rhIGF-I administration did not alter the expression levels of IGF-IR protein. Thus, the biological effect of circulating IGF-I on the tumors may indeed be mediated via the IGF-I receptor expressed on these tumors.

Previous studies have demonstrated that rhIGF-I administration to animals harboring tumors shortens the latency period for the appearance of microscopic tumors and enhances the growth rate of tumors that are already apparent in these animals (1, 2). Such studies led investigators to conclude that exogenous administration of IGF-I mediates tumor growth (1, 2). In the present study, rhIGF-I administration to both LID and control mice increased serum levels of IGF-I and subsequently increased the incidence of cecal tumor development, primary tumor size, and the number of mice demonstrating liver metastases. In the control mice, rhIGF-I treatment also increased the number of metastatic nodules observed. We propose that circulating IGF-I may be directly involved in tumor growth and metastases, at least in this model. On the other hand, there is no compelling evidence from these or other studies to suggest that the IGFs, IGFBPs, or their receptors are oncogenic, *i.e.*, capable of initiating tumor development.

Tumor growth is the outcome of two opposing forces: cellular proliferation, which increases cell number; and apoptosis, which reduces cell number. IGF-I, the IGFBPs, and the activated IGF-IR are ultimately involved in both of these processes. IGF-I enhances cellular proliferation mainly via activation of mitogen-activated protein kinase, phosphoinositide 3′-kinase, and other pathways, depending on the specific cell type. In all cell types, IGF-I enhances progression of the cell cycle. IGF-I can also exert its mitogenic effects by increasing the expression of mRNA or mRNA stabilization of potent growth factors, such as VEGF (17–19). On the other hand, IGF-I potently inhibits apoptosis, through the phosphoinositide 3′-kinase and mitogen-activated protein kinase pathways. Thus, a reduced level of circulating IGF-I in the LID mice may favor conditions that permit apoptosis and oppose the proliferation of tumor cells. Conversely, the administration of exogenous rhIGF-I could shift the equilibrium toward promoting cellular proliferation and opposing apoptosis. Additionally, the elevated serum IGFBP-3 is capable of prolonging the half-life of IGF-I in serum, thereby facilitating IGF-I bioavailability. With regard to the metastatic potential of these tumors, our results

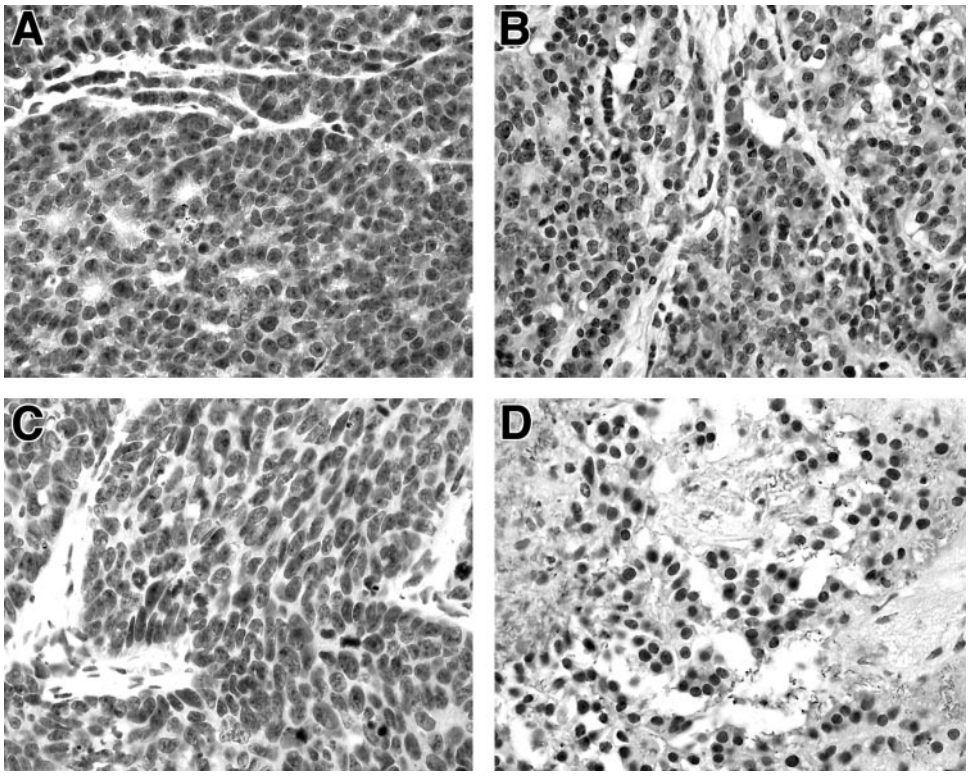

Fig. 3. VEGF expression in cecum tumor. VEGF immunoreactivity was evaluated in cecum tumors of control and LID mice that had been treated with either IGF-I or saline, as described in "Materials and Methods." *A*, control mice treated with IGF-I. The section shown is representative of that obtained from control mice, with an average intensity rating of 3+. *B*, control mice treated with saline. The section shown is representative of that obtained from control mice, with an average intensity rating of 2–3+. *C*, LID mice treated with IGF-I. The section shown is representative of that obtained from LID mice, with an average intensity rating of 1–2+. *D*, LID mice treated with saline. The section shown is representative of that obtained from LID mice, with an average intensity rating of 1+. Each section represents five to six mice in each group.

show clearly that there is a positive correlation between primary tumor size and the potential for development of metastases. Metastatic processes are still largely undefined. Local metastases along mechanical pathways may reflect migratory processes, whereas distant metastases are usually blood-borne and/or spread via the lymphatic system. Blood vessel formation within the tumors can permit malignant cells to enter into the general circulation, especially if the endothelium of tumor-associated vessels is defective. This may occur in central regions of the tumor in particular, where hypoxia or overt necrosis is more common. The angiogenic potential of a tumor is often an important factor in both the growth of the tumor and its ability to spread. Growth factors, such as angiogenin and VEGF, play a critical role in this process. VEGF is a hypoxia-regulated growth factor involved in angiogenesis and by implication, in tumor growth and metastases. Many tumor cell lines secrete VEGF *in vitro*, suggesting that this diffusible molecule is a mediator of tumor angiogenesis (20). VEGF mRNA is markedly up-regulated in the majority of human tumors including lung, breast, and gastrointestinal (21–25). Correlations have been documented between VEGF expression and microvessel density in primary breast cancer sections (26) and in gastric carcinoma patients (27). However, little is known about the role of VEGF in the process of tumor metastasis. Previous studies have demonstrated that IGF-I enhances VEGF gene expression. Therefore, it was of interest to investigate whether the expression of VEGF would be affected by the level of serum IGF-I and whether there is a correlation between the expression and metastasis of VEGF in our model. The cecal tumors from control mice in our study exhibited higher levels of VEGF as compared with tumors from LID mice. IGF-I injection was associated with an increased VEGF expression in tumors from both control and LID mice. These findings support reports of others in which IGF-I has been shown to induce VEGF expression in cultured colorectal carcinoma cells (18). Our data also indicated that the abundance of vessels in the cecum tumors correlated with the serum levels of IGF-I and the expression of VEGF. Because angiogenesis is associated with VEGF overexpression by tumor cells,

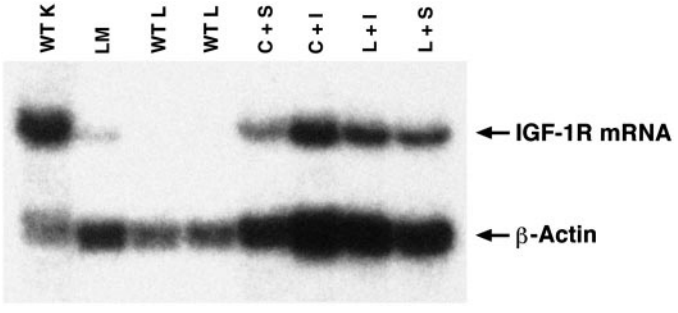

Fig. 5. IGF-IR mRNA is expressed in cecum tumors and in liver tissue containing metastatic nodules. RNase protection assays were used to determine IGF-IR mRNA levels in liver and kidney tissue from wild-type mice as well as cecum tumor and liver tissue containing metastatic nodules. *WTK*, wild-type kidney; *LM*, liver tissue containing metastatic nodule; *WTL*, wild-type liver; *C+S*, control, saline-treated; *C+I*, control, IGF-I-treated; *L+I*, LID, IGF-I-treated; *L+S*, LID, saline-treated. β-actin mRNA levels were used as a control for sample loading.

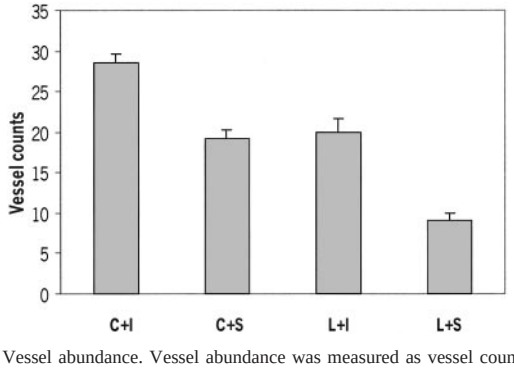

Fig. 4. Vessel abundance. Vessel abundance was measured as vessel counts (means; *bars*, SE; per ×100 field; $n = 5–6$ samples/group) using a factor VIII immunohistochemical staining technique.

we suggest that higher levels of VEGF would result in more vascularization in control mice as compared with LID mice. Higher levels of vascularization, in turn, presumably enable the robust metastatic process to occur as described previously (15). Thus, up-regulation of VEGF expression by IGF-I may influence tumor cell growth and metastases.

There is a possibility that the effects of IGF-I on tumor growth and metastases may be somehow related to an immunological response. IGF-I has been shown to play a role in proliferation and differentiation of cells from the immune system. Furthermore, Trojan *et al.* (28) demonstrated that C6 glioma cells expressing antisense IGF-I fail to grow in nude mice and also inhibit the growth of parental C6 glioma cells at a distance. It was suggested that this effect was attributable to an induced immune response via CD8+ lymphocytes. Could the lower levels of IGF-I in our LID mice confer an enhanced immunological response and thereby rejection of the tumor cells? Although this possibility must be considered, it remains entirely speculative at this point. We prefer a more straightforward interpretation of these results as a direct effect of IGF-I on activation of the IGF-IR, rather than an indirect effect on the immune system. Activation of the IGF-IR, in turn, would enhance cellular proliferation and inhibit apoptosis.

In summary, tumor growth and metastasis in mice are regulated by the levels of circulating IGF-I. Higher levels of IGF-I are associated with enhanced tumor development, and lower levels of IGF-I appear to diminish tumor development. The association of serum IGF-I levels with the risk for developing colon cancer in humans is therefore particularly significant, in view of the potential use of rhIGF-I as a therapeutic agent for a number of conditions including diabetes, renal failure, various catabolic syndromes, and age-associated tissue degeneration (29–32). In light of these issues, any attempts potentially enhancing serum levels of IGF-I must be approached with caution. However, strategies that are able to inhibit the function of IGF-I receptor by using IGF-IR specific antibody (33–34) or which are able to lower plasma levels of IGF-I by administrating of antagonists to the growth hormone-releasing hormone (35) or by implementing dietary interventions should be considered with the goal of preventing cancer.

ACKNOWLEDGMENTS

We thank Professor David E. Kleiner (NIH, Bethesda, MD) for reviewing the histology slides.

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